PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Robert De Leys
Bart Vanderborght
Eric Saman
Hugo Van Heuverswyn

Serial No.:

Filed: May 9, 2001

For: PROCESS FOR DETECTING HIV-3 RETROVIRUS (Amended)

Group Art Unit:

Examiner:

Atty. Dkt. No.:11362.0025.DVUS03
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PRELIMINARY AMENDMENT

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Please amend this application as follows:

IN THE ABSTRACT

Please add the following abstract on a separate page:

--Described is a new variety of retrovirus designated HIV-3, also known as HIV-1 subtype O, samples of which are deposited in the European Collection of Animal Cell Cultures (ECACC) under V88060301. Further described is a process to detect the HIV-3 retrovirus in biological liquids or tissue. One such process involves contacting a biological sample suspected of containing HIV-3 nucleic acids with a DNA probe corresponding to a segment of genomic HIV-3 retrovirus RNA, such as the LTR region, and detecting the hybridization products thereof.--

IN THE SPECIFICATION

On the title page, please delete the original title and insert a new title:

--PROCESS FOR DETECTING HIV-3 RETROVIRUS--

At page 1, line 1, insert a new first paragraph:

--This is a continuation of co-pending application Serial No. 09/379,270, filed August 23, 1999, which is a continuation of 08/900,902, filed July 25, 1997, now issued as United States Patent No. 6,013,484, which is a divisional of Serial No. 08/486,836, filed June 7, 1995, now issued as United States Patent No. 5,795,743, which is a divisional of Serial No. 08/228,519, filed April 15, 1994, now issued as United States Patent No. 5,567,603, which is a divisional of Serial No. 07/460,913, filed March 23, 1990, now issued as United States Patent No. 5,304,466, which claims benefit under 35 U.S.C. §120 of PCT/EP89/00643, filed June 8, 1989 and claims priority under 35 U.S.C. §119 of EP 88 109 200.1, filed June 9, 1988.--

At page 3, line 9, please insert a new paragraph:

--Subsequent to the filing of prior application Serial No. 08/228,519, the medical industry and scientific community has recognized the change in classification of HIV-3 to HIV-1 subtype 0. *See, e.g.,* Rayfield et al., *Emerging Infectious Diseases* 2:209-212 (1996); Janssens et al., *AIDS* 8:1012-1013 (1994); Simon et al., *AIDS* 8:1628-1629 (1994); Gürtler et al., *Journal of Virology* 68:1581-1585 (1994); and Vanden Haesevelde et al., *Journal of Virology* 68:1586-1596 (1994).--

On page 5, lines 24 – 33, please delete the two paragraphs and insert:

--Differential antigen capturing is performed as described hereinafter. The solid line represents the results obtained using a broad-spectrum anti-HIV-1 IgG while the broken line depicts the results obtained using an IgG which was rather specific for HIV-1. Figure 2A, Figure 2B, Figure 2C, Figure 2D, and Figure 2E each shows a typical titration obtained with HIV-1. Figure 2F shows the result obtained with HIV-3 (ANT 70) containing supernatant.

Figure 3A shows differential antigen capturing on HIV-1 and Figure 3B shows differential antigen capturing on HIV03 (ANT 70 NA) supernatants.--

On page 6, lines 7-8, please delete the paragraph and insert:

--Figure 4A shows the reactivity of anti-HIV sera on HIV-1 and Figure 4B shows the reactivity of anti-HIV sera on HIV-2 Western Blot strips.--

On page 7, lines 17-18, please delete the paragraph and insert:

--Figure 8A, Figure 8B, Figure 8C and Figure 8D show the effect of coating IgG dilution on the binding of HIV isolates.--.

On page 8, lines 6-7, please delete the paragraph and insert:

--Figures 10A-E show comparisons of reactivity of human anti-HIV antisera to different HIV types.--

On page 8, lines 19-30, please delete the three paragraphs and insert:

--Figures 11A-C show the titrations of anti-HIV sera by enzyme immunoassay.

Microwell plates were coated with lysates of HIV-1 (SF4), HIV-3 (ANT 70) and HIV-2 (isolate 53). Serum from an HIV-1-infected European (Fig. 11A), antiserum to HIV-3 (ANT 70 NA) (Fig. 11B) and antiserum to HIV-2 (isolate 53) (Fig. 11C) were titrated in 2-fold dilutions beginning at a dilution of 1:100 on all three coated plants.

Figures 12A and B show the positions of methionine and tryptophan residues in viral p17 and p24 gag gene products and Figure 12C shows the positions of methionine and tryptophan residues in viral pol gene products.--

On page 9, lines 11-25, please delete the three paragraphs and insert:

--Figures 13A-D show comparisons of partial cleavage products of gag and pol gene products of HIV-1 (SF4) [HIV-1 in the figure], HIV-3 (ANT 70) [isolate 70 in the figure], HIV-2rod [HIV-2 (LAV-2) in the figure] and HIV-2 (isolate 53) [isolate 53 in the figure]. The terms p24 and p17 are used in the genetic sense to indicate the largest and second largest viral core proteins, respectively.

Figures 14A-1, Figure 14A-II, Figure 14A-III, Figure 14B-1, Figure 14B-II, and Figure 14B-III show hybridization of cDNA clones to viral RNA.

Viral RNA from HIV-1 (SF4), HIV-2rod, and HIV-3 (ANT 70) were spotted onto a membrane filter as described in Materials and Methods. The filters were hybridized under either nonstringent (A) or stringent conditions (B) and autoradiographed.--

At page 55, lines 33- 37 please delete the paragraph and insert:

--The hybridization data also support the notion that ANT 70 is fundamentally different from either HIV-1 and HIV-2. As long as the conditions under which the hybridization is performed are stringent, a distinction can easily be made among the three virus types. RNA of the HIV-3 retrovirus virtually hybridizes neither with the Env gene or the LTR close to it, in particular not with the nucleotide sequence 8352-9538 of HIV-1, nor with the sequences of the Pol region of the HIV-1 genome under stringent conditions.--

IN THE CLAIMS

Cancel claims 1-30 and 32-36, without prejudice.

Please amend claim 31 to read:

31. (Amended) A process for the detection of HIV-3 retrovirus or of its RNA in a biological liquid or tissue, characterized by contacting nucleic acids contained in said biological liquid or tissue with a DNA probe containing at least 360 contiguous sequences corresponding to the genomic RNA of HIV-3 retrovirus under stringent hybridization conditions, washing the hybrid formed with a solution preserving said stringent conditions, and detecting the hybrid formed.

Please add claims 37-40 as follows:

--37. The process of claim 31 wherein the DNA probe is:

10	20	30	40	50	60
CCCATGGATT	TGAAGATACA	CATAAAGAAA	TACTGATGTG	GAAGTTTGAT	AGATCTCTAG
70	80	90	100	110	120
GCAACACCCA	TGTTGCTATG	ATAACTCACC	CAGAGCTCTT	CCAGAAGGAC	TAAAAACTGC
130	140	150	160	170	180
TGACCTGAAG	ATTGCTGACA	CTGTGGAACT	TTCCAGCAAA	GACTGCTGAC	ACTGCGGGGA
190	200	210	220	230	240
CTTTCCAGTG	GGAGGGACAG	GGGGCGGTTC	GGGGAGTGGC	TAACCCTCAG	AAGCTGCATA
250	260	270	280	290	300
TAAGCAGCCG	CTTTCTGCTT	GTACCGGGTC	TCGGTTAGAG	GACCAGGTCT	GAGCCCGGGA
310	320	330	340	350	360
GCTCCCTGGC	CTCTAGCTGA	ACCCGCTCGT	TAACGCTCAA	TAAAGCTTGC	CTTGAGTGAG

A

or the complement thereof.

38. The process of claim 31 wherein the DNA probe is:

10	20	30	40	50	60
AACATGGGAA	ACGCATTGAG	AAAAGGTAAA	TTTGAGGGAT	GGGCAGCAGT	AAGAGAAAGA
70	80	90	100	110	120
ATGAGAAGAA	CTAGAACTTT	CCCTGAGTCT	GAACCATGCG	CACCTGGAGT	AGGACAGATC
130	140	150	160	170	180
TCCAGGGAAT	TAGCAGCTAG	AGGAGGGATA	CCAAGTTCCC	ATACTCCTCA	AAACAATGCA
190	200	210	220	230	240
GCCCTTGCAT	TCCTAGAAAG	TCACCAAGAG	GAAGAAGTAG	GTTTTCCAGT	AGCACCTCAA
250	260	270	280	290	300
GTGCCTCTAA	GGCCAATGAC	CTATAAAGGA	GCATTTGACC	TCAGCTTCTT	TTTAAAAGAA
310	320	330	340	350	360
AAGGGAGGAC	TGGAAGGGTT	AATTTACTCC	CATAAAAGAG	CAGAAATCCT	GGATCTTTGG

GTGTATAA
or the complement thereof.

39. The process of claim 31 wherein the DNA probe corresponds to the nucleotide sequence coding for proteins p12, p16 or p25 of the HIV-3 retrovirus or the complement thereof.

40. The process of claim 31 wherein the DNA probe corresponds to the nucleotide sequence coding for glycoproteins gp41 or gp120 of the HIV-3 retrovirus or the complement thereof.--

REMARKS

The active claims in this case are claims 31 and 37-40.

The specification has been amended to recite the relationship with the parent cases, and to incorporate portions of the preliminary amendment filed August 23, 1999 in parent case 09/379,270. A marked up version of the amendments to the Specification is attached hereto. The original application was filed without an abstract of the disclosure. This Preliminary Amendment is being filed to provide such an abstract. The title has been amended to reflect the subject of the present claims.

The specification at page 3 has been amended to introduce the current classification, HIV-1 subtype O virus, for what had been termed "HIV-3" in the original application. After the inventors first reported on their discovery of HIV-3, specifically variant ANT_{70}, the medical and scientific community recognized that HIV-3 should more appropriately be classified as a subtype of HIV-1. This subtype was designated "O" where O stands for "outliers". Several journal articles have been provided in the preceding case Serial No. 08/486,836 to substantiate the scientific recognition that HIV-3, e.g., ANT_{70}, is now classified as HIV-1 subtype O.

The new claims find support at page 30, line 25 through page 32, line16; pages 37-40; pages 50-53; and original claims 25-31. A marked up version of the claim amendments is attached.

It is believed that no fee is due; however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Commissioner is authorized to deduct said fees from Deposit Account No. 01-2508/11362.0025.DVUS03.

Respectfully submitted,

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Date: May 9, 2001

MARKED-UP VERSION OF CLAIMS

31. (Amended) A process for the detection of HIV-3 retrovirus or of its RNA in a biological liquid or tissue, characterized by contacting nucleic acids contained in said biological liquid or tissue with a DNA probe containing [a nucleic acid according to any of claims 25 to 30] at least 360 contiguous sequences corresponding to the genomic RNA of HIV-3 retrovirus under stringent hybridization conditions, washing the hybrid formed with a solution preserving said stringent conditions, and detecting the hybrid formed.

--37. The process of claim 31 wherein the DNA probe is:

10	20	30	40	50	60
CCCATGGATT	TGAAGATACA	CATAAAGAAA	TACTGATGTG	GAAGTTTGAT	AGATCTCTAG
70	80	90	100	110	120
GCAACACCCA	TGTTGCTATG	ATAACTCACC	CAGAGCTCTT	CCAGAAGGAC	TAAAAACTGC
130	140	150	160	170	180
TGACCTGAAG	ATTGCTGACA	CTGTGGAACT	TTCCAGCAAA	GACTGCTGAC	ACTGCGGGGA
190	200	210	220	230	240
CTTTCCAGTG	GGAGGGACAG	GGGGCGGTTC	GGGGAGTGGC	TAACCCTCAG	AAGCTGCATA
250	260	270	280	290	300
TAAGCAGCCG	CTTTCTGCTT	GTACCGGGTC	TCGGTTAGAG	GACCAGGTCT	GAGCCCGGGA
310	320	330	340	350	360
GCTCCCTGGC	CTCTAGCTGA	ACCCGCTCGT	TAACGCTCAA	TAAAGCTTGC	CTTGAGTGAG

A

or the complement thereof.

38. The process of claim 31 wherein the DNA probe is:

10	20	30	40	50	60
AACATGGGAA	ACGCATTGAG	AAAAGGTAAA	TTTGAGGGAT	GGGCAGCAGT	AAGAGAAAGA
70	80	90	100	110	120
ATGAGAAGAA	CTAGAACTTT	CCCTGAGTCT	GAACCATGCG	CACCTGGAGT	AGGACAGATC
130	140	150	160	170	180
TCCAGGGAAT	TAGCAGCTAG	AGGAGGGATA	CCAAGTTCCC	ATACTCCTCA	AAACAATGCA
190	200	210	220	230	240
GCCCTTGCAT	TCCTAGAAAG	TCACCAAGAG	GAAGAAGTAG	GTTTTCCAGT	AGCACCTCAA
250	260	270	280	290	300
GTGCCTCTAA	GGCCAATGAC	CTATAAAGGA	GCATTTGACC	TCAGCTTCTT	TTTAAAAGAA

310 320 330 340 350 360
AAGGGAGGAC TGGAAGGGTT AATTTACTCC CATAAAAGAG CAGAAATCCT GGATCTTTGG

GTGTATAA
or the complement thereof.

39. The process of claim 31 wherein the DNA probe corresponds to the nucleotide sequence coding for proteins p12, p16 or p25 of the HIV-3 retrovirus or the complement thereof.

40. The process of claim 31 wherein the DNA probe corresponds to the nucleotide sequence coding for glycoproteins gp41 or gp120 of the HIV-3 retrovirus or the complement thereof.--

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^HIV-3 RETROVIRUS ~~AND ITS USE~~ PROCESS FOR DETECTING

Inventors: Robert De Leys
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source of virus.

Furthermore, the invention relates to a purified retrovirus having the essential morphological and immunological properties described below. In many cases, the unique characteristics of HIV-3 can best be appreciated by comparison with the same type of characteristics relating to the other human immunideficiency viruses, HIV-1 and HIV-2.

Brief description of the drawings

In Figures 1 to 16 the designations HIV-3 (ANT 70) and HIV-3 (ANT 70 NA) refer to two strains of a new HIV-3 virus isolated from a Camerounian woman and her partner from which HIV-3 (ANT 70) has been deposited under ECACC V88060301.

Figure 1 shows a procedure for preparing cleavage maps of viral proteins.

Figure 2 shows differential antigen capturing on virus-containing culture supernatants.

Differential antigen capturing is performed as described hereinafter. The solid line represents the results obtained using a broad-spectrum anti-HIV-1 IgG while the broken line depicts the results obtained using an IgG which was rather specific for HIV-1. ~~The titrations shown in panels A-E are typical for HIV-1. Panel F~~ shows the result obtained with HIV-3 (ANT 70) containing supernatant.

Figure 3A shows differential antigen capturing on HIV-1 and HIV-3 (ANT 70 NA) supernatants.

Figure 3B shows differential antigen capturing on Differential antigen capturing was performed as described hereinafter. The solid line depicts the results obtained on

Figure 2A, Figure 2B, Figure 2C, Figure 2D, and Figure 2E each show a typical titration obtained with HIV-1. Figure 2F

plates coated with the broad spectrum anti-HIV IgG while the broken line represents the results obtained on plates coated with IgG which shows less crossreactivity with HIV types other then HIV-1.

Figure 4A shows the reactivity of anti-HIV sera on HIV-1 and HIV-2 Western Blot strips.
↑ Figure 4B shows the reactivity of anti-HIV sera on
The reactivities of 3 different sera on HIV-1 and HIV-2 Western blot strips are shown. Sera: 1. anti-HIV-1, 2. anti-HIV-3 (ANT 70), 3. anti-HIV-2 (isolate 53). The molecular weights indicated are those given by the manufacturer (Dupont Biotech).

Figure 5 relates to the comparison of gag and pol proteins of several HIV-1 isolates, HIV-2rod and HIV-3 (ANT 70).

Proteins were separated electrophoretically and blotted as described later. The blot was incubated with a broad-spectrum anti- HIV antiserum followed by (anti-human IgG)/alkaline phosphatase- labeled conjugate to visualize the proteins.
A. HIV-2rod, B. an HIV-1 laboratory isolate, C. HIV-3 (ANT 70), D. an HIV-1 laboratory isolate, E. HIV-1 (SF4).

Figure 6 shows a comparison of HIV-3 (ANT 70) and HIV-3 (ANT 70 NA) proteins.

Proteins were separated electrophoretically and blotted as described later The blot was incubated with the BSR antiserum followed by (alkaline phosphatase)/anti-human IgG conjugate to visualize the proteins. Lane 1: HIV-3 (ANT 70 NA), lane 2: HIV-3 (ANT 70), lane 3: HIV-1 (SF4). The apparent intensity difference between lanes 1 and 2 is caused by the difference in the amount of material loaded.

Figure 7 relates to the ability of various human anti-HIV-1 sera to capture viral antigens.

A number of human sera were diluted 1:1000 and coated directly on microwell plates. Detergent-treated culture supernatants containing HIV-1 (SF4), HIV-3 (ANT 70), HIV-2rod or HIV-2 (isolate 53) were incubated and the bound antigen was detected using a broadspectrum (anti-HIV)/ horseradish peroxidase conjugate. Sera 1-7 were of African origin while sera 8-11 were from Europeans. The greater ability of African sera to capture non-HIV-1 antigen can, in part, be explained by their higher anti-p24 titers (data not shown).

A, Figure 8B, Figure 8C and Figure 8D show Figure 8, ~~shows~~ the effect of coating IgG dilution on the binding of HIV isolates.
Succesive 2-fold dilutions were made of four different sera, beginning at a dilution of 1:1000 and were used to coat microwell plates. Detergent-treated supernatants of HIV-1 (SF4), HIV-3 (ANT 70), HIV-2rod and HIV-2 (isolate 53) were diluted to give approximately the same optical density on plates coated with the antiserum shown in panel B at a dilution of 1:1000. Bound antigen was detected using the broad-spectrum (anti-HIV IgG)/horseradish peroxidase conjugate.

Figure 9 shows antigen capturing of virus isolates using human polyclonal and mouse anti-HIV-1 monoclonal antibodies.

Wells were coated and incubated as described in the text. The IgGs used are as follows:

1. human polyclonal anti-HIV IgG , 2. MAb CLB 59, 3. MAb CLB

21, 4. MAb CLB 64, 5. MAb CLB 14, 6. MAb CLB 16, 7. MAb CLB 47, 8. MAb CLB 13.6 (anti-p18), 9. MAb CLB 19.7, 10. Mab CLB 13.4 (anti-p18).

Figure 10 ~~is a comparison~~ A-E show comparisons of the reactivity of human anti-HIV antisera to different HIV types.

Lysates of HIV-1 (SF4), HIV-3 (ANT 70), HIV-2rod and HIV-2 (isolate 53) were separated electrophoretically on SDS-polyacrylamide gels, blotted onto nitrocellulose, and incubated with a high titer anti-HIV-1 antiserum (panel A), a lower titer anti-HIV-1 antiserum (panel B), serum from the woman from whom HIV-3 (ANT 70) was isolated (panel C), her partner from which HIV-3 (ANT 70 NA) was isolated (panel D) and anti-HIV-2 antiserum from the person from whom HIV-2 (isolate 53) was isolated (panel E).

Figure 11 ~~shows~~ A-C show titrations of anti-HIV sera by enzyme immunoassay.

Microwell plates were coated with lysates of HIV-1 (SF4), HIV-3 (ANT 70) and HIV-2 (isolate 53). Serum from an HIV-1-infected European (~~left panel~~ Fig. 11A), antiserum to HIV-3 (ANT 70 NA) (~~center panel~~ Fig. 11B) and antiserum to HIV-2 (isolate 53) (~~right panel~~ Fig. 11C) were titrated in 2-fold dilutions beginning at a dilution of 1:100 on all three coated plates.

Figure 12 ~~shows~~ A and B show the positions of methionine and tryptophan residues in viral gag and pol gene products. p17 and p24 gene products. Amino acid positions for the p17 gag proteins are given starting from the first methionine in the coding sequence. Positions for the p24 gag protein are given starting at the p17/p24 proteolytic cleavage site. Positions for the pol gene are shown after alignment with the highly conserved

and Figure 12C shows the positions of methionine and tryptophan residues in viral

tryptophan doublet in the HIV-1 sequence at positions 556 and 557. The positions of a conserved protease sequence, the protease/reverse transcriptase cleavage site and the reverse transcriptase/endonuclease cleavage site are indicated. In this case, the terms p24 and p17 are used in the genetic sense to refer to the largest and second largest viral core proteins respectively. The term "HIV-2 (LAV-2)" is a synonymum for HIV-2 rod.

Figures 13A-D show comparisons, ~~Figure 13 is a comparison~~ of partial cleavage products of gag and pol gene products of HIV-1 (SF4) [HIV-1 in the figure], HIV-3 (ANT 70) [isolate 70 in the figure], HIV-2rod [HIV-2 (LAV-2) in the figure] and HIV-2 (isolate 53) [isolate 53 in the figure]. The terms p24 and p17 are used in the genetic sense to indicate the largest and second largest viral core proteins, respectively.

Figures 14A-I, Figure 14A-II, Figure 14A-III, Figure 14B-I, Figure 14B-II, and Figure 14B-III show ~~Figure 14 shows~~ hybridization of cDNA probes to viral RNA.

Viral RNA from HIV-1 (SF4), HIV-2rod, and HIV-3 (ANT 70) were spotted onto a membrane filter as described in Materials and Methods. The filters were hybridized under either nonstringent (A) or stringent conditions (B) and autoradiographed.

1. Morphology

Electron microscopy of HIV-3-infected MT4 cells revealed the presence of extracellular virus particles having a diameter

antigenic differences between ANT 70 and HIV-1 are smaller than those between HIV-2 and HIV-1. This is particularly evident from the results presented in Figures 8 and 10.

Additional compelling evidence that ANT 70 is a unique virus different from HIV-1 and HIV-2 comes from the partial peptide maps. We have shown that there are significant differences in the most highly conserved viral proteins. The two HIV-2 isolates which were used for comparison gave essentially identical cleavage patterns except in the case of CNBr cleavage of the p17 core protein. It should be noted, however, that the p17 core protein exhibits more variability than the p24 protein, at least in HIV-1 strains (34). Whether or not this also holds true for HIV-2 awaits sequence determination on more strains than have been analyzed to date.

In light of the fact that ANT 70 is antigenically more closely related to HIV-1 than is HIV-2, as evidenced by a higher degree of crossreactivity which extends even to the gp41 envelope protein, was essential to establish that ANT 70 was more than simply a genetic variant of HIV-1. This was possible by investigating the locations of some of the most highly conserved amino acids in a number of viral proteins which are least subject to genetic variation. That major differences were noted in the cleavage patterns indicates that HIV-1, HIV-2 and ANT 70 are three genetically distinct viruses. On the other hand, the same series of experiments also revealed similarities between the viruses which may indicate that all three arose from a common progenitor.

The hybridization data also support the notion that ANT 70 is fundamentally different from either HIV-1 and HIV-2. As long as the conditions under which the hybridization is performed are stringent, a distinction can easily be made among ~~between~~ the three virus types. RNA of the HIV-3 retrovirus virtually hybridizes neither with the Env gene or the LTR close to it, in particular not with the nucleotide sequence 8352 - 9538 of HIV-1, nor with the sequences of the Pol region of the HIV-1 genome under stringent conditions.